PROGRESS REPORT

TO THE

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

Research Grant NGR-05-020-137: Structure and Function of Proteins and Nucleic Acid

Semi-annual Report for the Period January 1, 1967 to June 30, 1967

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1. Publications

a. Stryer, L., Holmgren, A., and Reichard, P., Thioredoxin. A Localized Conformational Change Accompanying Reduction of the Protein to the Sulfhydryl Form, Biochemistry 6, 1016 (1967).

b. Haugland, R.P. and Stryer, L., A Fluorescent Probe at the Active Site of α-Chymotrypsin, in "Conformation of Biopolymers," ed. by G.N. Ramachandran, Volume 1, pp. 321-335 (1967).

c. Stryer, L. and Haugland, R.P., Energy Transfer: A Spectroscopic Ruler, Proc. Natl. Acad. Sci. U.S. 58, 719 (1967).

2. Energy Transfer: A Spectroscopic Ruler

The most important finding of the past year is that the efficiency of electronic energy transfer can be used to measure distances between 10 and 60 Å. The energy transfer process was studied in a system of defined length. An α-naphthyl energy donor group and a dansyl energy acceptor group were attached to the ends of oligomers of a helical polypeptide, poly-L-proline. Oligomers containing between 1 and 12 proline units served as spacers of defined length from 12 to 46 Å. In this distance range, the transfer efficiency decreased from close to 100% to 16%. It was 50% efficient at 35 Å. The transfer efficiency falls off as the inverse sixth power of the distance between the energy donor and acceptor, as predicted by theory. This is the first direct experimental confirmation of the theory of energy transfer. A particularly interesting possibility is that the energy donor-acceptor pairs can be used to reveal proximity relationships in biological macromolecules. Are two amino acids in a protein near or far apart? Work is in progress using energy transfer to map the three-dimensional structure of a protein in solution. The method requires specific labeling of proteins with fluorescent groups. We have accomplished this in the case of α-chymotrypsin, in which a single fluroescent group has been inserted at the active site. We are now developing a series of selective fluorescent labeling reagents which will be useful in energy transfer studies of protein structure.

3. Nanosecond fluorescence studies of protein mobility.

An important biological question is whether proteins are rigid or flexible structures. When proteins bind substrates, is there a structural change or is the binding site preformed? Our nanosecond fluorimeter is being used to investigate the rotational mobility of proteins. The polarization of the fluorescence emitted by a group at the active site of the enzyme is measured as a function of time in the nanosecond time range. The emission anisotropy, A, is found to be an exponential function of time, $A = A_o e^{-t/\phi}$, where $A_o$ is the anisotropy at o time, and $\phi$ is the rotational relaxation time. A long rotational relaxation time indicates that the active site is rigid, while a short relaxation time would show that it is flexible. For $\alpha$-chymotrypsin, the first enzyme we have investigated, we find a markedly rigid active site. This suggests that the protein has a well-defined structure which does not change in times shorter than a microsecond. We plan to investigate other proteins and nucleic acids in this way to determine whether the rigidity of structure found for chymotrypsin is a general characteristic of biological macromolecules. The nanosecond system is now under computer control.

4. Fluorescence microscopy of protein crystals.

A sensitive fluorescence microscope has been constructed. Protein crystals are being studied. By looking at the fluorescence intensity as a function of angle, we can obtain information concerning the orientation of fluorescent groups in the protein. This type of angular information is not available in studies of protein fluorescence in solution, since the samples are unoriented. We hope to gain insight into the details of intermolecular interactions by having this directional data.

5. Kinetic properties of mutant hemoglobins.

The binding of azide ion to two abnormal hemoglobins was studied. The reaction is of interest since myoglobin, a one-subunit protein, shows a fast reaction, while hemoglobin, a four-subunit protein, shows a slow reaction. The question arises as to whether this difference between myoglobin and hemoglobin arises from subunit interactions. In fact, one of the mutant hemoglobins, Hb $M_{Iwate}$, behaved like myoglobin, demonstrating that the binding kinetics of the $\beta$-subunit depend on whether the adjacent subunit is normal or mutant.